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Transmission of immunoglobulin to foetal and neonatal mice

P. Appleby and D. Catty

Department of Immunology, The Medical School, University of Birmingham, Birmingham, B15 2TJ, U.K.

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Transmission of immunoglobulin (Ig) classes and subclasses from mother to foetus and to neonate, and the survival of maternal Ig in the circulation of the young mouse up to 40 days after birth, has been quantitated in Balb/c homozygous and (Balb/c×SJL/J)F₁ matings using isotype-specific heteroantisera in radial immunodiffusion in gel assays. The transfer of anti-allotype (anti-Ig-1^b(γ2a)) antibodies from immunised Balb/c mothers (Ig-1^{ab}) to F₁ heterozygote (Ig-1^{ab}) offspring was measured by passive haemagglutination of Ig-1^b target allotype-coated sheep red blood cells. A small but significant level of transmission of Ig to the foetus occurs by the 15th day of gestation (5 days before birth) but the bulk of passively acquired Ig is derived from the milk after birth. All Ig acquired in utero and later across the intestinal barrier is exclusively of IgG isotypes (γ1, γ2a, γ2b) even though the milk has a large predominance of IgA. An appreciable level of maternally derived antibody is maintained in the circulation of the young mouse 24 days or more after gut 'closure' on the 16th day post-partum.

Introduction

The transmission of passive immunity from mother to young has been extensively studied in several species, but particularly in the rat and rabbit (Brambell, 1970). The mouse has not received an equivalent degree of attention, which is surprising in view of its importance as a laboratory experimental animal, particularly for immuno- logical investigations. The usefulness of the mouse Ig allotypic markers, coupled with the observed consequences of transmitted anti-allotype antibodies, has stimulated us to obtain basic quantitative and qualitative data on transmission of isotypes and of specific antibody. This complements our other work in the system which examines transmission of identified antibody clones from mother to young (Appleby and Catty, 1980), and the role of transmitted allo-antibodies on differentiation of B cells in the neonate (in preparation).

Early work by Malkinson (1967) had demonstrated that some degree of resistance to vaccinia virus in the mouse could be acquired in utero but the major period of transmission of resistance occurred after birth. Carretti and Ovary (1969) were then able to show that a proportion of the antibodies transmitted before birth were in the γ1 and γ2 isotypes. Such transmission occurs presumably across the placenta or yolk sac endoderm, or both. Fahey and Barth (1965) were the first to demonstrate the quantitative importance of antibody transmission from milk after birth in the mouse,

a route that had been clearly defined by Halliday in 1959, who had shown that absorption of antibody by the proximal small intestine continued to the 16th day post-partum. Fahey and Barth showed that there was a rapid acquisition of IgG by neonates from maternal milk but no maternal IgA or IgM could be found in neonatal serum, even though IgA, at least, is present in high concentration in the milk.

Materials and Methods

Mice

SJL/J, C57BL/10, CB20 (all Ig-1^b allotype) and Balb/c (Ig-1^a) were obtained from the mouse colony of the Department. Balb/c female mice immunised against the Ig-1^b allotype (see below) were trial bled and their sera tested by gel diffusion. Only those showing a single precipitin line with sera of SJL/J and a strong precipitin band with the purified γ2a myeloma protein of PC101 (CB20 origin) were selected for mating.

Antisera

Sheep anti-mouse Ig isotype sera were raised by immunisation in Freund's Complete Adjuvant (FCA), with mouse myeloma proteins purified by DEAE cellulose chromatography from the following secreted plasmacytoma products: IgA (MOPC 315), IgM (MOPC 104), γ1 (MPC 21), γ2a (APC 5), γ2b (MPC 11). The plasmacytomas were all kindly donated by Dr. D. Dresser of the National Institute for Medical Research. The antisera were rendered specific by absorption with glutaraldehyde cross-linked (insoluble) purified myeloma proteins or the poly-merised lysate material of extensively washed myeloma cells.

Balb/c anti-Ig-1^b serum was prepared according to the method of Herzenberg and Herzenberg (1973) and was affinity-purified using a Sepharose-PC101 (γ2a, Ig-1^b) immunoadsorbent. All antisera were tested for specificity by gel diffusion.

Radial immunodiffusion in gel assays

These were performed on 8 × 8 cm glass plates of 1% barbitone agarose, pH 8.6, containing 2% (w/v) polyethylene glycol (PEG, BDH, 6000 daltons). Volumes of 1 or 2 μl of whole serum to be quantified for isotype were applied to 2.7 mm diameter wells in the agarose gel, using a Hamilton syringe. Precipitin ring diameters were measured after 24 and 48 h at room temperature and the test samples compared with standards included on each plate. Results are expressed as a percentage of the corresponding normal adult serum pool.

Passive haemagglutination

This was performed in Cooke round-bottomed microtitre plates using 1% foetal calf serum (FCS) in phosphate-buffered saline (PBS) as diluent. Sheep red blood cells (SRBC) were coated with purified γ2a protein of the PC101 mouse myeloma, obtained from the Ig-1^b homozygous CB20 allotype congenic strain of Balb/c origin.

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The coating method was that of Goding (1976) using $CrCl_3$. Sensitised cells were run against standard alloantisera as a control in each assay plate to ensure uniformity in the titration of antibody.

Serum samples

Adult mice were bled routinely from the tail. Foetal and neonatal serum was obtained by sacrifice and heart puncture. Foetal serum samples from individual litters were pooled, owing to the small volume of serum obtained from each foetus. Amniotic fluid, bathing the foetus, was collected by foetal sacrifice. All sera and amniotic fluids were stored at $-20°C$. Milk was drawn from lactating Balb/c mothers under anaesthesia following subcutaneous injection of 3 I.U. synotocin. Milking was accomplished using a miniaturised suction apparatus. 200–300 μl of milk were obtained from each mouse; this was centrifuged at 10000 rev./min $(14000 \times g)$ for 10 min and the clear supernatant collected and stored at $-20°C$.

Results

Transmission of maternal immunoglobulins according to class and subclass into the amniotic fluid (AF) and foetal serum (FS) in Balb/c and (Balb/c × SJL/J)F_1
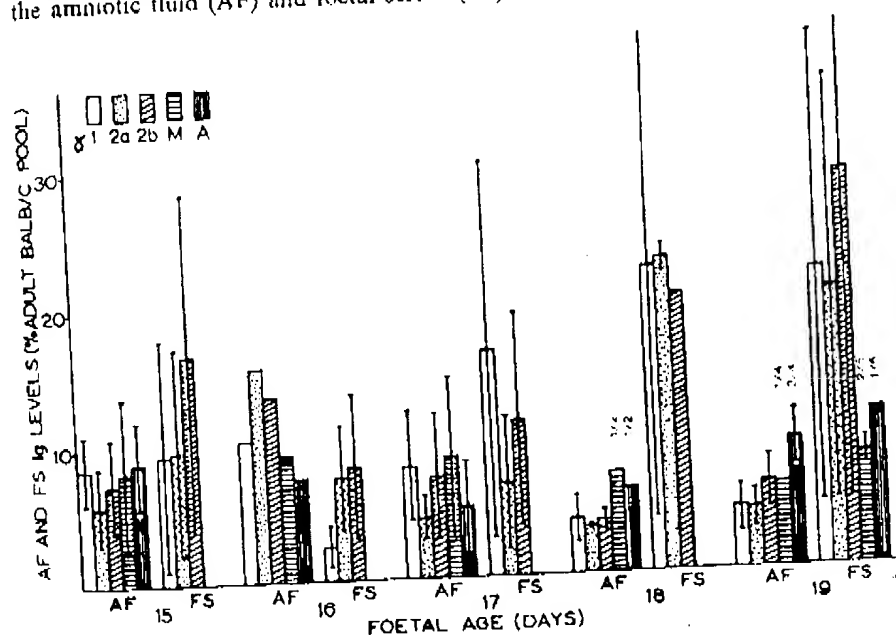

Fig. 1. Amniotic fluid (AF) and foetal serum (FS) immunoglobulin (Ig) class and subclass levels at days 15–19 of gestation in the mouse expressed as a percentage of the levels of the respective class or subclass in an adult Balb/c serum pool. Standard errors are shown where samples are not pools and two or more results are used. Fractions above the columns refer to number of samples that are positive for the relevant isotype in the total group of samples tested, i.e. one out of 6 (1/6), etc.

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pregnancies is shown in Fig. 1. Maternal Ig of the γ1, γ2a and γ2b subclasses can be found regularly in the foetal circulation on day 15 of gestation in concentrations around 10–15% of that of adult Balb/c serum. Day 15 is the earliest practicable gestational age to obtain the required quantity of foetal blood but from the levels of Ig present at this time it is evident that transmission must have begun some days earlier. Tests showed that serum Ig from 15 days to birth (and beyond) is entirely of maternal origin as (Balb/c × SJL/J)F₁ foetal serum contains no Ig carrying the paternal allotypic markers. Amniotic fluid harvested from the space around the foetus on day 15 of gestation (and probably before) also contains low levels of maternally derived immunoglobulins of the γ1, γ2a and γ2b subclasses, but in addition IgM and IgA, which are excluded from the foetus. The concentrations of the IgG isotypes in foetal sera varied considerably between litters although in every case each subclass of IgG is represented and there is no obvious selection of subclass for transmission to the foetus. A progressive rise in the levels of all IgG subclasses in foetal serum can be seen from the 15th day to term (19th day). Mean concentrations of IgG subclasses at birth are 20–30% of those of adult mice, and thus a significant quantity of passively acquired IgG antibodies is available before suckling commences, to confer protection upon the otherwise defenseless neonate. At term a small proportion of mice tested (10–20%) contained low levels of serum IgM and IgA. This is thought to be of maternal origin since these classes of Ig are never detectable
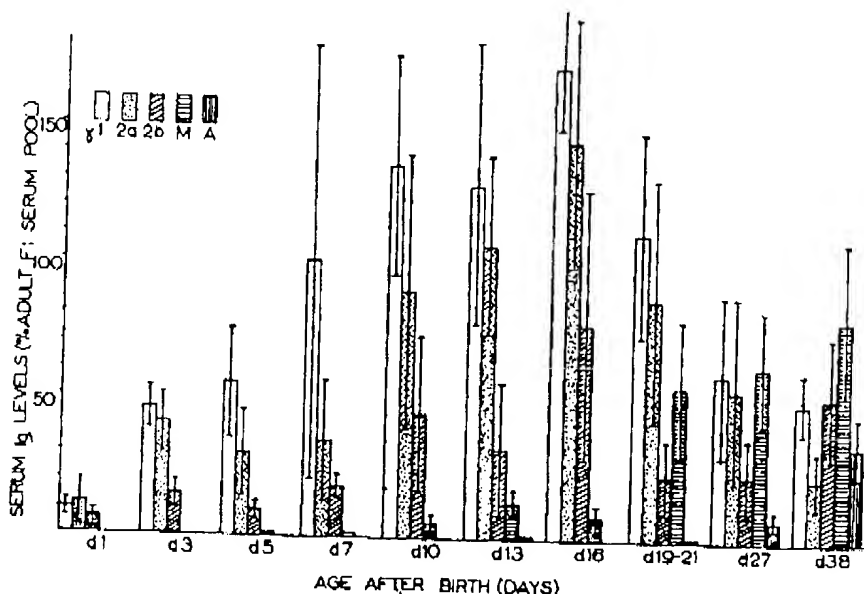

Fig. 2. Serum immunoglobulin class and subclass levels in (Balb/c × SJL/J) F₁ mice from day 1 to day 38 after birth expressed as a percentage of the levels of the respective class or subclass in an adult F₁ serum pool. Standard errors are shown except where only single samples are positive. 5 or more neonates from separate litters were sacrificed for each sample group.

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in neonatal serum in the first five days after birth and do not reach equivalent levels
to those seen sporadically at term before 10–30 days after birth when autologous
synthesis is under way (Fig. 2).

The transmission of Ig to the suckling mouse and its persistance in serum after
gut 'closure' is shown in Fig. 2. In this study, 5 or more neonates from separate
litters were sacrificed for each sample group. The data presented show the rapid
acquisition of maternal immunoglobulin in large quantities in the first two weeks
after birth, followed by a gradual decline after day 16 to a condition of transient
physiological hypogammaglobulinaemia at 4–5 weeks of age. One day after birth
neonatal serum Ig levels are substantially lower than in the last few days of foetal
life, but with the onset of transmission via the gut, IgG levels rise dramatically and
peak at 16 days. There is an obvious early selection in favour of γ1 antibodies which
reach the adult level in the first week and then consistently exceed this level for two
further weeks. The γ2a concentration rises progressively to reach the adult level and
above in the second week after birth; serum γ2b also increases in concentration
throughout the transmission period but more slowly than the other two IgG
subclasses; it only reaches adult levels, and then only transiently, in the third week
and the level falls dramatically after gut 'closure' through an assumed more rapid
catabolic rate and short half-life. All passively acquired IgG shows a decline in
concentration after transmission stops on the 16th day, as catabolism is no longer

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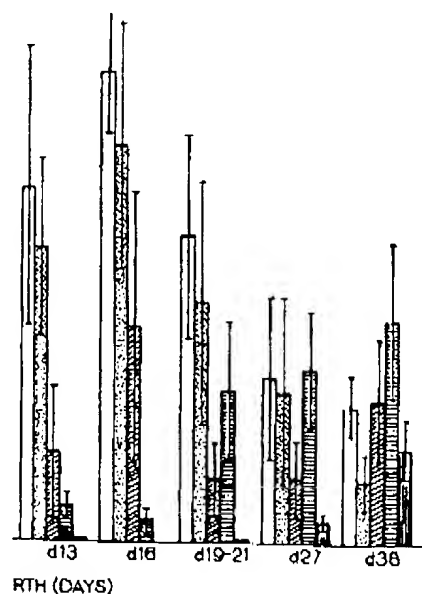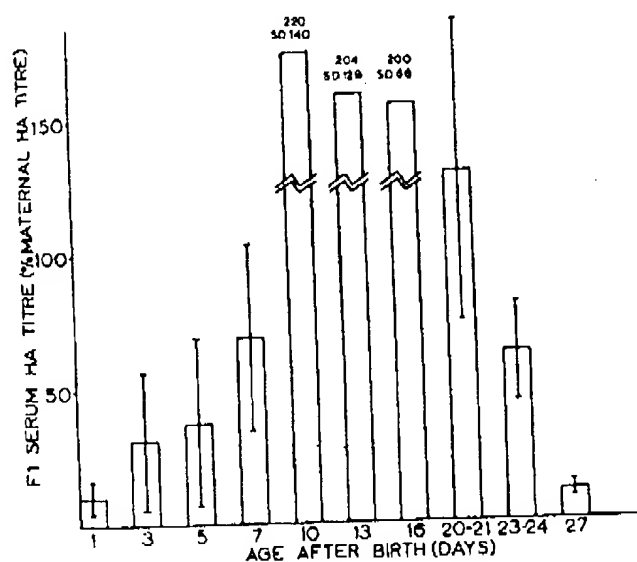
Fig. 3. Serum anti-allotype (anti-Ig-1ᵇ) antibody (passive haemagglutination) levels in (Balb/c × SJL./J)F₁
mice from day 1 to day 27 after birth, expressed as a percentage of their maternal haemagglutination
(HA) titres, with standard deviations (S.D.) shown. Each column represents the mean of a minimum of
four mice taken from separate litters on the days shown. The results on days 10, 13 and 16 are shown by
the figures (with S.D.) above the columns.

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compensated by uptake of milk antibodies. IgM is not transmitted across the gut
wall of suckling mice but is the first autologous immunoglobulin of the neonate to
appear in the serum of some individual mice at day 5 and in all mice from day 13
onwards. From the 3rd week after birth the serum level of IgM begins to rise
progressively towards adult levels, which are reached in some mice in the 6th week.
The appearance of autologous serum IgA does not occur regularly in the young
mouse until the 4th week and the level then rises only slowly over the next few
weeks. The first IgG subclass to show evidence of autologous synthesis was γ2b
whose concentration also began to rise between the 4th and 5th weeks after birth.
The transmission and synthesis of γ3 was not examined in our studies.

The pattern of transmission of total IgG from the milk to the neonatal circulation
is followed to a large degree, as might be expected, in the appearance and titre of
specific anti-allotype antibodies transmitted from immunised Balb/c mothers to
their heterozygous offspring (Fig. 3). The results, expressed as a percentage of the
respective maternal antibody titre, are the mean of a minimum of four mice taken
from separate litters on the days shown. At birth, antibody obtained by transmission
in utero is only 10% of the titre of the maternal serum, but the concentration rises
rapidly over 10 days and the suckling mouse is able to accumulate antibody by a
highly efficient absorption from the gut of milk IgG, to such an extent that the 10
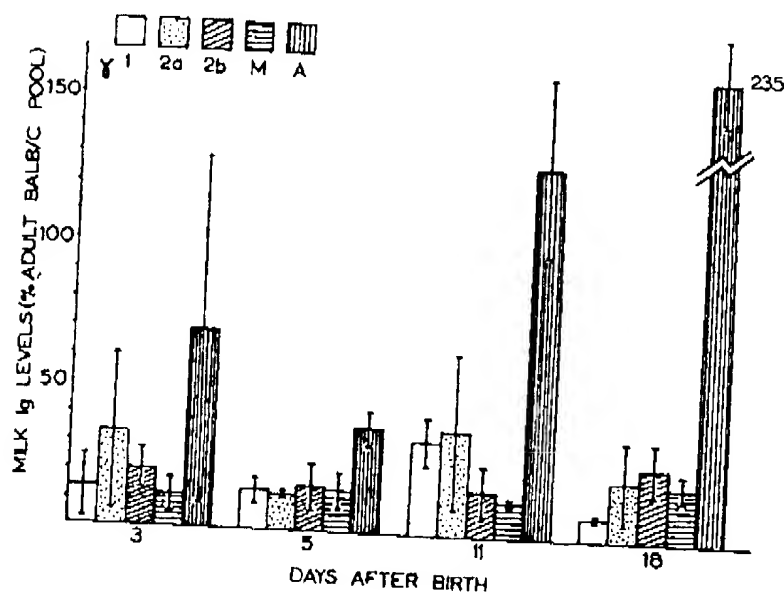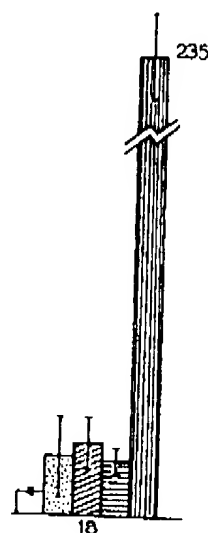day old mouse has antibody titres at least twice those in the maternal serum. This

Fig. 4. The levels of immunoglobulin (Ig) classes and subclasses in the milk of lactating Balb/c mice from
3 to 18 days after parturition, expressed as a percentage of the levels of the respective class or subclass in
an adult Balb/c serum pool. Standard errors are shown for each result. Samples were taken from only 2
or 3 mothers on each day so as not to prejudice the transmission results, shown elsewhere (Fig. 2).

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condition is sustained for two weeks but upon gut 'closure' antibody levels fall
dramatically over the next two weeks owing to the short average catabolic half life of
mouse IgG (between 3 and 4 days based on our data). Antibody at less than 10% of
maternal titre persists to the 27th day after birth and in some animals traces of
antibody can be detected as late as days 35–40. No differences could be detected
between (Balb/c × SJL/J)F₁ and (Balb/c × C57BL/10)F₁ heterozygote offspring in
their ability to take up and concentrate maternal anti-Ig-1[b] antibody from the
mothers' milk.

A further aspect of our investigation of antibody transmission involved the
quantitation of immunoglobulins in milk taken from the Balb/c mothers. The
results of this study are presented in Fig. 4. It was possible to take only a limited
number of samples from 2 or 3 lactating mothers on the days shown. However, it is
clear that the level of all immunoglobulins in milk, except IgA, is low and varies
little during the whole suckling period; IgG and IgM levels are consistently about
20% of the concentrations in normal adult serum. However, IgA in milk is, by the
11th day of lactation, very substantially above the adult serum level. The amount of
IgA appears to increase during suckling and at the time of natural weaning the
concentration is at least double that of adult serum. It is also interesting to note that
beyond day 16 milk continues to contain IgG, even though the young mouse is
unable to absorb it intact across the gut wall, i.e. after gut 'closure'. Antibody levels
are not shown in Fig. 4 but the anti-allotype titre of milk antibodies has been
measured and regularly achieves levels approximately half of those of the maternal
serum by day 11.

Discussion

It is clear from our results that the transmission of Ig from mother to young
mouse begins relatively early in the second half of the gestational period, probably
2-3 days before the earliest age at which we were able to obtain foetal blood samples,
and continues well into the third week of post-natal life. Morphis and Gitlin (1970)
described a materno-foetal IgG transport mechanism in the mouse which becomes
active between 11 and 15 days of gestation, and some prenatal antibody transmis-
sion studies done by ourselves, but not presented here, show that anti-SRBC activity
can be detected, at very low levels, as early as day 14 of gestation. These results
support the findings of Malkinson (1967), who demonstrated the protective effect of
prenatally transmitted antibody to vaccinia virus in mice, and indicate that the foetal
mouse is the recipient of rather higher levels of maternal Ig than had been previously
noted (Bell et al., 1966). In our studies foetal serum Ig often reached a level in excess
of 25% of that found in the maternal circulation at term and the importance of this
prenatal transmission must not be underestimated.

The route of transmission in utero in the mouse has not been investigated
thoroughly, although Brambell and Halliday (1956), working on transmission of
maternal immunoglobulin in the rat, proposed three possible routes: two of these
involve uptake of immunoglobulin from the uterine lumen, one directly into the

foetal circulation by way of the yolk sac and vitelline circulation, and the other by absorption across the foetal gut after ingestion of amniotic fluid, the immunoglobu. lin having passed across the yolk sac and amnion from the uterine lumen. A transplacental passage of immunoglobulin was also implicated on the basis of experiments which showed transmission to the foetus even after surgical interference with the vitelline circulation. It is unfortunate that samples of uterine fluid from pregnant mice are so difficult to obtain after 10 days of gestation due to the close apposition of the conceptus to the uterine wall. From our investigations it is clear, however, that the amniotic fluid bathing the foetus contains immunoglobulins of the IgG, IgM and IgA classes which are of maternal origin. We find no evidence that young mice can synthesise any substantial amount of their own IgM or IgA until more than one week (IgM) or 3 weeks (IgA) after birth. The amniotic fluid IgG carries only the maternal allotype (of γ2a) in situations where matings have given rise to F_1 Ig-1^{ab} heterozygote conceptuses. How the maternal immunoglobulin arrives in the amniotic fluid has not been adequately explored but, because it is there in considerable amount from at least the time that Ig is found in foetal serum, it must be regarded as a possible source for transmission to the foetus by the vitelline route or across the foetal gut wall after ingestion. It can be noted that amniotic fluid contains more or less equivalent amounts of γ1, γ2a and γ2b subclasses of IgG (Fig. 1). These all reduce in concentration in the last 3 days of gestation. The dramatic increase in these same subclasses in the foetal circulation at this time is the best available evidence for an amniotic fluid–foetal transmission. Should this occur across the foetal gut wall then we do not see, prior to birth, the gut epithelium imposing the same IgG subclass restrictions on transport as it apparently does after birth in dealing with maternal milk-derived antibodies (Fig. 2).

Although not examined in this study, there is considerable evidence that immune IgG3 may be selectively transmitted across the placenta prior to birth, often reaching concentrations in excess of those found in the maternal circulation (Gitlin 1971, Grey et al, 1971). However, postnatally, Guyer et al (1976) could demonstrate only minimal specific binding of IgG3 to intestinal Fc receptors of the neonatal gut, indicating that the bulk of transmission of IgG3 may occur prior to birth. It has been shown that IgG3 constitutes the major isotype in antibody responses to common bacterial carbohydrate antigens (Perlmutter et al, 1978) and this may explain the reported high level of prenatal transmission. However, whether IgG3 is selectively transmitted postnatally clearly remains to be determined.

The initial fall in neonatal IgG levels following birth may be the result of a number of factors, such as an immature IgG transport mechanism in the neonatal gut or low levels of Ig in the first colostral secretions of the mother. It was not possible to test this second hypothesis, as milking immediately post-partum would have jeopardised the litters. Serum loss or other trauma associated with parturition may induce a transient hypogammaglobulinaemia which persists for just 1–2 days until the neonate begins actively to concentrate maternal Ig taken from the milk. The mechanism of milk Ig absorption has been investigated in rodents by a number of workers, using mainly the rat, and results have shown the importance of Fc receptors found on the surface of cells lining the proximal small intestine (Jones and

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ial small intestine (Jones and

Waldmann, 1972; Rodewald, 1976; Borthistle et al., 1977). A similar mechanism is assumed to operate in the intestine of suckling mice. Our results clearly demonstrate that an IgG sub-class-specific selection mechanism governs the uptake and transmission of IgG by the neonatal gut; the IgG1 sub-class is the most efficiently transmitted, with IgG2a being only slightly less readily taken up, while IgG2b is only poorly transmitted. Guyer et al., (1976) reported similar findings. Neither these workers nor ourselves could find any evidence to indicate that IgM or IgA were selectively transported to the neonate, although according to Jones (1976) some absorption of intact molecules may occur in the more distal regions of the small intestine.

In the mouse the preferential uptake of IgG1 and IgG2a to the neonatal blood could be expected on the grounds that, as they are the isotypes of choice synthesised by adults, they would be expected to have a priority role in the protection of the neonate. This may be an explanation for their more efficient transmission across the gut wall. IgG1 constitutes 60% of the adult total serum IgG and IgG2a makes up another 20–30%. Thus IgG2b and IgG3 can only make up the remaining 10–20% of serum Ig (Natsuume-Sakai et al, 1977; Potter, 1972). It is reasonable to assume that the required protective properties of passive antibody are adequately represented in the two predominant IgG sub-classes under most circumstances.

Because of the great efficiency of transport of IgG across the gut wall there is no demand for very high levels of IgG in the gut lumen. Indeed, the low level of IgG found in the milk, compared with IgA, may well be of advantage to both the mother and the neonate; high levels of IgG would keep the transport mechanism saturated with a resulting unnecessary wastage of unbound IgG through proteolysis (Waldmann and Jones, 1976; Morris, 1976). Surprisingly, secretion of IgG into milk is sustained even in samples taken after gut 'closure' on day 16 even though by this time it can be of no further use as a transmissible protective antibody. Presumably it can continue to perform an important role in protecting the gut from infection until weaning. IgA is the grossly predominant immunoglobulin class to be found in milk throughout lactation, however, and there is no doubt that it has the primary role as protective antibody in external secretions (Tomasi and Bienenstock, 1968).

Although we did not test for anti-Ig-1[b] activity in immunoglobulin isotype fractions of the immune milk (because of the small volumes collected), the anti-Ig-1[b] titre of the milk was at least half that of the maternal serum. Since milk contains relatively lower levels of IgG than are present in the maternal serum we must assume that some anti-Ig-1[b] antibody resides in the IgA fraction as well as the IgG fraction. Stechschulte and Austen (1970), however, could not detect anti-hapten activity in milk IgA of rats after systemic immunisation with DNP-BGG, although IgG antibody activity was easily demonstrated. That substantial amounts of anti-Ig-1[b] activity reside in the IgG fraction of the milk in our model also is obvious as this is the only source of such antibody obtainable by the neonate (i.e. in the circulation). Comparison of Figs. 2 and 3 clearly shows a correlation between rising serum IgG levels and anti-Ig-1[b] titre.

The gut of the neonatal mouse remains permeable to the passage of intact IgG until 16 days, after which the uptake of maternal antibody abruptly ceases (Halliday,

1959; Brambell, 1970). Our results are in agreement with this finding as both antibody titre and neonatal immunoglobulin levels, which are maximal between days 10 and 16, begin to decline rapidly after day 16. The catabolism of immunoglobulin, like transmission, is postulated to be an Fc-related phenomenon (Fahey and Robinson, 1963; Brambell, 1966), and the lower level of transmission, and higher catabolic rate, of IgG2b would be in keeping with a mechanism in which IgG molecules compete for a limited number of Fc receptors. It can thus be postulated that these mouse Fc receptors, involved in catabolism and transmission, have a higher affinity for IgG1 and IgG2a than for IgG2b, and, presumably, IgG3, although this requires further clarification.

Acknowledgements

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